

# Northumbria Research Link

Citation: Brasier, A.T., Rogerson, Michael, Mercedes-Martin, R., Vonhof, H.B. and Reijmer, J.J.G. (2015) A Test of the Biogenicity Criteria Established for Microfossils and Stromatolites on Quaternary Tufa and Speleothem Materials Formed in the "Twilight Zone" at Caerwys, UK. *Astrobiology*, 15 (10). pp. 883-900. ISSN 1531-1074

Published by: Mary Ann Liebert, Inc.

URL: <https://doi.org/10.1089/ast.2015.1293> <<https://doi.org/10.1089/ast.2015.1293>>

This version was downloaded from Northumbria Research Link:  
<http://nrl.northumbria.ac.uk/id/eprint/44845/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)



UniversityLibrary



1    **A Test of the Biogenicity Criteria Established for**  
2    **Microfossils and Stromatolites on Quaternary Tufa and**  
3    **Speleothem Materials Formed in the “Twilight Zone” at**  
4    **Caerwys, U.K.**

5  
6    A.T. Brasier<sup>1\*</sup>, M.R. Rogerson<sup>2</sup>, R. Mercedes-Martin<sup>2</sup>, H.B. Vonhof<sup>3</sup> and J.J.G.  
7    Reijmer<sup>3</sup>.

8    1\*: Corresponding Author: School of Geosciences, Meston Building, University  
9    of Aberdeen, Old Aberdeen, Scotland, UK. AB24 3UE. Email:  
10    [a.brasier@abdn.ac.uk](mailto:a.brasier@abdn.ac.uk); Telephone: +44(0)1224 273 449; Fax: +44(0)1224 278  
11    585.

12    2: Department of Geography, Environment and Earth Science University of  
13    Hull Cottingham Road, Hull, UK. HU6 7RX

14    3: Faculty of Earth and Life Sciences, VU University Amsterdam, De Boelelaan  
15    1085, 1081HV, Amsterdam, The Netherlands

16

17    **Running title:** Biogenicity of tufa stromatolites

19    **Abstract**

20    The ability to distinguish the features of a chemical sedimentary rock that can  
21    only be attributed to biology is a challenge relevant to both geobiology and  
22    astrobiology. This study aimed to test criteria for recognizing petrographically the  
23    biogenicity of microbially influenced fabrics and fossil microbes in complex  
24    Quaternary stalactitic carbonate rocks from Caerwys, UK. We found that the  
25    presence of carbonaceous microfossils, fabrics produced by the calcification of  
26    microbial filaments, and the asymmetrical development of tufa fabrics due to the  
27    more rapid growth of microbially influenced laminations could be recognized as  
28    biogenic features. Petrographic evidence also indicated that the development of  
29    "speleothem-like" laminae was related to episodes of growth interrupted by  
30    intervals of non-deposition and erosion. The lack of any biogenic characteristics  
31    in these laminae is consistent with their development as a result of variation in the  
32    physico-chemical parameters that drive calcite precipitation from meteoric waters  
33    in such environmental settings.

34    **Key words:** microfossil, stromatolite; biogenicity; carbonate; tufa; speleothem

35

## 36 **Introduction**

37 The ability to distinguish those features of a chemical sedimentary rock that can  
38 only result from biology has proven to be a challenge for carbonate deposits (e.g.,  
39 Wright and Barnett, 2015). As such an aim is essential for astrobiology and  
40 geobiology research (e.g., Cady and Noffke, 2009), we tested the ability to  
41 recognize signs of life and identify fossilized microbiota in Quaternary tufas and  
42 speleothem materials with the use of biogenicity criteria developed to recognize  
43 ancient microfossils (Sugitani et al., 2007; Wacey, 2009) and stromatolites (Buick  
44 et al., 1981; Hofmann, 2000). Of particular relevance to life detection in  
45 carbonates is the issue of a boundary that has been somewhat arbitrarily drawn  
46 between “speleothem” terrestrial carbonate rocks that form in dark caves,  
47 typically consisting of coarse columnar crystals that grow at rates of up to around  
48 100 microns per year and are assumed to be essentially abiotic (e.g., Fairchild et  
49 al., 2006; Frisia and Borsato, 2010), and spring and stream “tufa” carbonate rocks.  
50 The latter are very similar to speleothem carbonate rocks, but they form in spring,  
51 stream and lake settings where photosynthetic cyanobacteria, algae and plants  
52 abound (e.g., Andrews and Brasier, 2005; Brasier et al., 2010) and are most  
53 commonly comprised of small “micritic” calcite crystals. Unlike speleothems,  
54 tufas are increasingly presumed to be biotically influenced (e.g., Freytet and  
55 Verrecchia, 1998; Arp et al., 2001; Pedley, 2014). Tufas grow more rapidly than  
56 speleothems, at rates of several millimeters to centimeters per year. There is

57 increasing recognition that tufa and speleothem systems form part of a continuum  
58 that also includes hydrothermal “travertines” (Rogerson et al., 2014), which  
59 suggests that some aspects of speleothem growth are likely to be enhanced by  
60 microbial growth in some instances (Cacchio et al., 2004).

61

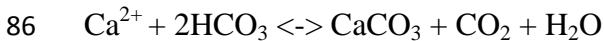
62 To test whether existing biogenicity criteria for microfossils and stromatolites  
63 could distinguish biotic from abiotic features in chemical sedimentary  
64 precipitates, we applied them to the potentially complex specimens that grow in  
65 the so-called “twilight zone” at the entrance to modern caves (see Jones, 2010),  
66 and in caverns of Quaternary tufa deposits. Carbonate rocks that formed in a  
67 twilight zone where there is a transition from tufa to speleothem carbonate rocks  
68 include the Holocene barrage tufa deposit located at Caerwys, north Wales, UK  
69 (Figs. 1 and 2; Pedley, 1987). This tufa deposit contains several meter-scale  
70 cavities or caverns that are locally decorated with speleothem-like stalactitic  
71 calcite. The cavities are a primary feature that formed behind “curtains” of  
72 petrified moss that draped over tufa dams known as “barrages” (Figs. 2 and 3).  
73 We examined whether characteristics of the fabrics of specimens that formed in  
74 these twilight zone conditions could be differentiated from those formed biotically  
75 under the influence of photosynthesisers (“tufa-like”), and those formed  
76 abiotically in the dark (“speleothem-like”). We also determined which

77 petrographic criteria developed for distinguishing biotic from abiotic precipitates  
78 were most successful when applied to these Holocene specimens, with potential  
79 implications for early Earth studies and astrobiology searches.

80

81 *Abiotic vs biotic origins of tufas and speleothems*

82 Calcite precipitation in streams where tufas form was, until recently, most  
83 commonly viewed as a purely physico-chemical process (e.g., Zhang et al., 2001)  
84 in which CO<sub>2</sub> outgasses from fluids causing them to become supersaturated with  
85 respect to calcite, as reflected in the net thermodynamic equilibrium equation:



87 CO<sub>2</sub> gas enrichment in meteoric waters occurs where they filter through biogenic  
88 CO<sub>2</sub>-rich soil zones. The partial pressure of CO<sub>2</sub> builds in these environments so  
89 modern soil zone pCO<sub>2</sub> commonly considerably exceeds that of atmospheric CO<sub>2</sub>.  
90 Calcite precipitation (Eq. 1) is typically associated with outgassing of this gas-rich  
91 fluid at spring effluents and where such fluids flow over obstacles like boulders or  
92 tree roots (e.g., Zhang et al., 2001). Recently this model has been challenged by  
93 Hammer and co-authors (2010) who found that dissolved gas concentrations  
94 decrease very little by turbulence caused by flow over surface irregularities,  
95 which led them to conclude that the most important role of turbulence was to

96 increase precipitation rates by bringing solutes to and from the calcite surface  
97 during turbulent mixing in the water column. Though the formation of stalactite  
98 and stalagmite speleothems in caves has most commonly been attributed to  
99 abiotic calcite precipitation driven by such physico-chemical processes (e.g.,  
100 Frisia and Borsato, 2010), field studies (Freytet and Verrecchia, 1998; Vazquez-  
101 Urbez et al., 2009; Glunk et al., 2011) and laboratory experiments with viable  
102 biofilms collected from modern stream settings (Rogerson et al., 2008; Pedley et  
103 al., 2009) have demonstrated that both abiotic and biotic processes influence  
104 calcite precipitation in streams.

105 Recent experiments and field studies have also demonstrated that tufa  
106 precipitation can be significantly enhanced, even driven, by microbial  
107 extracellular polymeric substances (EPS) (e.g., Rogerson et al., 2008; Glunk et al.,  
108 2011; Pedley et al., 2014). The EPS of non-viable organisms has also been  
109 implicated in tufa formation (Rogerson et al., 2008). Biofilms of some species of  
110 cyanobacteria are reportedly associated with their own specific microfabrics  
111 (Freytet and Verrecchia, 1998), and such petrographic evidence would support the  
112 hypothesis that some cyanobacteria exert direct control on tufa formation.

113

114 Diagenetic alteration following deposition adds further complexity to the ability  
115 to determine the biogenicity of precipitated carbonate rocks like tufas and



116 speleothems. In spring or stream carbonate deposits, primary biofabrics may be  
117 rapidly overprinted and recrystallized generating textures that appear abiotic  
118 (Jones and Peng, 2012). For example, diagenetic changes transform primary tufas  
119 from finely crystalline (micritic) to more coarsely crystalline (sparry) calcite, the  
120 fabric changing either by aggrading neomorphism (Love and Chafetz, 1988;  
121 Janssen et al., 1999) or simply continued growth of favored crystals (Brasier et  
122 al., 2011). Yet characterizing all coarse, sparry calcite fabrics as either alteration  
123 products or abiotic would be an over-simplification (Brasier et al., 2011) that  
124 could lead to many microbial fossils, both ancient and modern, being overlooked.

125

## 126 **Materials**

### 127 *The Caerwys tufa*

128 The tufa deposit studied here was found to the south of the village of Caerwys in  
129 north Wales, UK (Figs. 1 and 2). The waters that formed the tufa were sourced  
130 from a spring in the Carboniferous limestone (Pedley, 1987). Tufa precipitation at  
131 Caerwys has been constrained via radiocarbon techniques to the Holocene and  
132 pre-Holocene ‘Late Glacial’ intervals (Preece and Turner, 1990). Deposition has  
133 now ceased but a small analogous active site is found nearby at Ddol (Preece and  
134 Turner, 1990). The Caerwys tufa forms a veneer of Quaternary terrestrial

135 carbonate rock over the underlying geology. It grew in a steep stream that flowed  
136 down the scarp-face of a Carboniferous Limestone outcrop, with a series of  
137 cascading pools developing behind arcuate tufa dams or “barrages” that were  
138 oriented transverse to flow (Fig. 2; Pedley, 1987). The Caerwys system would  
139 have resembled the currently active tufa-depositing stream at Alport, Derbyshire,  
140 UK (Fig. 3). In both cases (Caerwys and Alport) unconsolidated micrite muds are  
141 found in the pools between the barrages. Some of these pools contain isolated  
142 decimeter-scale thrombolitic heads constructed by calcifying cyanobacteria, green  
143 algae, and invertebrates. The barrage dams themselves comprise indurated but  
144 vuggy carbonate walls that include a similar assemblage of fossil organisms.  
145 Though extensively quarried, the internal fabric of the barrage system is visible  
146 (Fig. 2) and preserved as a site of special scientific interest (SSSI).

147

148 Lithostratigraphy and mollusc biostratigraphy of the quarry were described by  
149 Preece (1978; 1982) and a facies model was compiled by Pedley (1987). Tufa  
150 oxygen and carbon isotopes were detailed and their Quaternary climate  
151 implications discussed by Garnett et al. (2006). The latter estimated summer  
152 water temperatures were in the range of 13 to 16.5 °C on the basis of tufa and  
153 gastropod  $\delta^{18}\text{O}$  values.

154

155 *Specimens exhibiting the continuum of tufas and speleothems*

156 Pedley (1990) described calcitic tubes of tufa formed by fringe cements that  
157 encrusted larger plants (macrophytes). The samples analyzed in this study fit that  
158 basic description and calcite encrustations of macrophytes are identified by their  
159 morphology. Internally they have a central longitudinal cavity that may remain  
160 open or may have been filled by clastic grains (commonly but not necessarily of  
161 tufa carbonate) or calcite cement. They are differentiated from hollow abiotic  
162 stalactites such as “soda straws” in that they contain preserved carbonaceous  
163 matter or recognizable impressions of organisms that become encrusted when  
164 mineralized. In swampy paludal marshes and pool margins, most plant stems  
165 grow upwards and, when encrusted by carbonate, become stalagmite-like deposits  
166 (Pedley, 1990). In barrage systems, however, many plants including bryophytes  
167 live on the overhangs of the barrage (dam) front, their stems and branches  
168 hanging downwards. This leads to pendant, stalactite-like calcite-encrusted  
169 structures (Fig. 2). Externally these calcitic encrustations appear indistinguishable  
170 from “speleothem” stalactites or stalagmites formed in caverns. Encrustations  
171 may be <10 mm to several tens of centimeters in width, and range from a few  
172 centimeters to meters in length. Such encrustations are common but under-  
173 reported for modern karst settings. The oldest known examples may be the  
174 pendant cavity-filling cements of the 2.75 Ga Fortescue Group, Australia, which  
175 were interpreted as biogenic by Rasmussen et al. (2009).

176

## 177   **Methodology**

178   Eleven different tubular specimens from Caerwys, each several centimeters in  
179   length, were collected. All were examined in hand specimen and using standard  
180   petrographic microscopy techniques. Specimens were injected with blue resin,  
181   and both longitudinal and transverse thin-sections were prepared and analyzed.  
182   All rocks were found to be minimally or non-luminescent when viewed under UV  
183   light. Cathodoluminescence (CL) microscopy revealed that none of the specimens  
184   luminesced. Thin-sections were examined and photographed with the use of  
185   polarized light microscopy. Carbonaceous filaments entombed in calcite were  
186   distinguished from dark micritic calcite by partially dissolving the specimen with  
187   acetic acid. Similar petrographic histories were elucidated from all eleven  
188   specimens, such that we could select two representative specimens, labelled S3.2  
189   and Caerwys 1, for detailed study.

190

## 191   **Results**

192   The descriptions of hand-specimen scale observations are followed by  
193   microscopic observations for specimen S3.2, followed by the same for specimen  
194   Caerwys 1. The terms micrite (crystals <4µm diameter), microspar (4 to 10µm

195 diameter), and spar ( $>10\mu\text{m}$  diameter) are used to indicate crystal sizes and do not  
196 reflect the origin of the grains.

197

198 *Specimen S3.2: Hand-specimen scale observations*

199 Specimen S3.2 is a downward-tapering stalactitic rock (Fig. 4) that was collected  
200 as a float specimen that was found adjacent to the central barrage outcrop in the  
201 quarry (Fig. 2b and 2c). Internally there is strong asymmetry, and at least six  
202 separate components of the visible fabric of the sample, referred to here as Zones  
203 1-6, were delineated (Fig 5).

204 Zone 1 is located in the central section of the porous tufa, which is permeated by  
205 c. 50 to 100  $\mu\text{m}$  diameter hollow tubes of calcite that form shrub-like growths  
206 oriented downward at around 55 degrees from horizontal (Fig. 4b). From  
207 examination of longitudinal and transverse sections it was possible to discern that  
208 these growths formed via micritic calcite encrustation of a biological (likely  
209 cyanobacterial) substratum. The asymmetrical nature of the sample, when viewed  
210 in cross-section, is due to the more extensive outward growth of this primary  
211 depositional fabric in one direction away from the point of initiation. Denser-  
212 looking white patches of tufa, developed on one (likely the upstream) side of this  
213 sample, cover an area around 3 mm across and a centimeter in length.

214 Zone 2 (Fig. 5) consists of micrite intergrown with dark brown sparry calcite fans,  
215 with the latter being dominant. Each fan is around 1 to 3 mm across, and all fans  
216 grew toward the outward edge of the specimen. This zone forms a band around 1  
217 cm thick on one side of the specimen (the side toward which Zone 1 micrite  
218 prograded), and is traceable continuously around the perimeter, though it narrows  
219 to <1 mm thick on the opposite side of the sample.

220 Zone 3 in hand-specimen consists of a 1 to 2 mm thick light brown band that  
221 envelops calcite of Zone 2, and is capped by a darker brown 1 mm thick band.  
222 Internally these bands contain several very fine sub-mm thick cream colored  
223 laminae. These bands are thicker where they developed below cm-scale  
224 overhangs.

225 Zone 4 is recognizable in hand-specimen only as a white band that separates  
226 brown calcite below it from very similar-looking brown calcite above, which is  
227 designated as Zone 5. Note that Zone 4 appears brown in thin-section (Fig. 5).

228 Zone 6, the outer casing of the specimen, consists of a layer of cream colored  
229 botryoidal calcite, around 2 mm thick on one side and 6 mm thick on the other.  
230 This outer zone seems to be divided into two bands by a very thin dark lamina.

231

232 *Specimen S3.2: Microscopy*

233 Two thin-sections of sample S3.2 were made from the cut specimen. Scans of  
234 these are shown in Figure 4, one from the transverse sawn section (S3.2A) and the  
235 other from the longitudinal sawn section (S3.2B). Petrographic analysis of these  
236 thin sections confirmed that Zone 1 comprises 6.5 to 26  $\mu\text{m}$  (mostly c. 20  $\mu\text{m}$ )  
237 diameter dominantly sub-vertically oriented biological filaments that were  
238 encrusted by 18 to 50  $\mu\text{m}$  thick walls of calcite (Figs. 4, 5 and 6a). The  
239 carbonaceous filaments themselves have mostly been oxidized, leaving behind  
240 empty calcite tubes (Figs. 6a and 7a), though some tubes contain carbonaceous  
241 material that resembles hollow and partially decomposed cyanobacterial  
242 trichomes within a sheath (Figs. 7b and c). The central parts of each calcite tube  
243 that would have been in contact with the carbonaceous filament are comprised of  
244 an envelope of small microcrystalline crystals. The remaining outer part of each  
245 calcite tube wall is constructed of radiating c. 20  $\mu\text{m}$  diameter spar crystals (Fig.  
246 7a). Also in Zone 1 is a cluster of hollow calcite tubes that ranged from 250 to  
247 500  $\mu\text{m}$  across (Fig. 7d). These each have an inner zone of microspar (crystals c.  
248 20  $\mu\text{m}$  diameter) followed by two rings of larger sparry crystals, each c. 45  $\mu\text{m}$   
249 thick. Some of these tubes have sparry calcite fan growths on one side, which  
250 marks the start of Zone 2.

251

252 The first Zone 2 sparry calcite fans (Figs. 4, 5 and 6a) nucleated directly on the  
253 spar of Zone 1 encrusted tubes. These two spar types are, in some locations, in  
254 optical continuity, which indicates that Zone 1 spar acted as a template for  
255 precipitation and growth of Zone 2 spar. Some curved, dark micritic growth lines  
256 within the fans are traceable between fans (Fig. 6a). Fans interfered with each  
257 other where they touched during growth. Some fans terminated in smooth curved  
258 surfaces; others were flat-topped (e.g. Fig. 8); and several exhibited pointed  
259 euhedral crystal terminations. Growth of Zone 2 seemingly ceased for extended  
260 periods of time (perhaps months?) on at least three occasions. These cessations  
261 are marked by dark micritic layers from which new fans nucleated. The latter are  
262 indicated by their different crystallographic orientations from the underlying fans  
263 (Fig. 6a). A magnified view of one of the Zone 2 fans, shown in Fig. 8, reveals  
264 that the carbonaceous filaments interpreted as entombed cyanobacteria are  
265 dominantly but not exclusively oriented sub-parallel to the direction of crystal  
266 growth. They are c. 2  $\mu\text{m}$  wide and 40  $\mu\text{m}$  long, distinctly narrower than the  
267 carbonaceous filaments of Zone 1. These narrower filaments cross through some  
268 of the finer,  $\mu\text{m}$  scale crystal growth bands within the fans (Fig. 8). The  $\mu\text{m}$  scale  
269 growth bands were likely formed on approximately diurnal timescales (Andrews  
270 and Brasier, 2005). Pedley (1987) suggested spar-entombed carbonaceous  
271 filaments of the Caerwys quarry could be *Schizothrix* or *Phormidium* sp.  
272 cyanobacterial fossils on the basis of growth form and diameter.



273

274 The contact between Zones 2 and 3 of this specimen (Figs. 4, 5, 6 and 9) is sharp  
275 in places though more transitional in others due the optical continuity of  
276 subsequent crystal growth. At its base, Zone 3 comprises numerous (at least six)  
277 couplets of micrite and columnar sparite (Fig. 9b). A micrite band c. 5  $\mu\text{m}$  thick  
278 forms the nucleation region for numerous crystals of spar (mostly c.100  $\mu\text{m}$   
279 diameter) in several locations in the specimen. Petrography revealed that several  
280 of the latter columnar spar crystals, commonly with length to width ratios  $>6$ ,  
281 developed through competitive growth of crystals that nucleated in this band (e.g.,  
282 Figs. 9c and 9d). Other columnar crystals stem from lower horizons that include  
283 Zone 2 spar crystals (e.g., Fig. 9a). Inclusions in the columnar crystals appear as  
284 bands oriented perpendicular to the direction of growth and parallel to (and  
285 commonly in close proximity to) the bands of micrite (e.g., Fig. 9c and 9d). The  
286 inclusions were identified primarily within the calcite crystals rather than between  
287 them.

288 Most intriguing near the top of Zone 3 (Figs. 4, 5 and 10) is a patch a few mm  
289 across that includes a series of circular to oval pores that range from 100 to 240  
290  $\mu\text{m}$  in diameter (Fig. 10). The latter are most easily interpreted as transverse  
291 cross-sections of hollow tubes. One tube oriented longitudinally in the thin-  
292 section extends for at least 2.2 mm. At higher magnification it was determined

293 that the walls of these tubes are thinly lined with dark micrite, surrounded by  
294 cylinders comprised of spar crystals 100 to 400  $\mu\text{m}$  in diameter (Fig. 10c and  
295 10d). The latter are differently aligned from the columnar calcite host, radiating  
296 outward from the tube center. The crystals aligned parallel to the direction of  
297 growth of the columnar spar are elongated upward, forming flame-like growth  
298 shapes. These crystals evidently grew contemporaneously and in competition with  
299 the surrounding columnar spar.

300 Zone 4 was recognized petrographically by the re-appearance of micrite on one  
301 side of the specimen (Fig 5b). This zone is laterally traceable into spar via two c.  
302 5  $\mu\text{m}$  thick sub-parallel micritic bands that mark its top and bottom. The micrite  
303 comprises a porous network of peloids that includes hollow tubes of c. 130  $\mu\text{m}$   
304 diameter.

305 Zone 5 spar crystals terminations range from pointed to flat or broken and are  
306 delineated by inclusions (see Fig 5 and Fig. 11). In places they are draped in  
307 continuous bands of dark, dust-like micrite that infilled the depressions and  
308 smoothed out the topography (Fig. 11a). Most spar crystals terminate at these  
309 micrite layers, though this was not always the case (Fig. 11b). For example, in one  
310 place a fragment of micrite 340  $\mu\text{m}$  across adhered to the specimen surface (Fig.  
311 11) and sits flat on a micritic layer. Spar crystals nucleated on this detrital micrite  
312 inclusion, though they were later out-competed by other columnar crystals.

313 Further, the protrusion formed by this blob of micrite clearly affected  
314 development of the overlying layers (Fig. 11).

315 The tops of Zone 5 columnar crystals formed the substrate for Zone 6 crystal  
316 growth. Zone 6 crystals (Fig. 5 and Fig. 12) are distinctly different from those of  
317 Zone 5 and appear as networks of needle-like laths. The latter are arranged along  
318 the faces of a crystal lattice (Figs. 12a and 12b). These laths are sub-crystals  
319 (crystallites *sensu* Kendall and Broughton, 1978) that range individually in size up  
320 to c. 1 mm long and 50  $\mu\text{m}$  wide and that link to form larger millimeter-to-  
321 centimeter scale composite crystals (Frisia and Borsato, 2010). These composite  
322 crystals are sometimes in optical continuity with the Zone 5 columnar calcite on  
323 which they grew (Fig. 12).

324

#### 325 *Specimen Caerwys 1: Hand-specimen scale observations*

326 Caerwys 1 is an 8 cm long specimen that grew in a stalactitic fashion. It was  
327 collected from a cavern within *in situ* barrage deposits located in the center of the  
328 quarry. This specimen was collected from directly adjacent to the much larger  
329 stalactitic rock shown in Fig. 2c. In longitudinal section it was possible to  
330 recognize the sample has a highly porous center surrounded by several c. 0.5 to 1  
331 mm thick layers that alternate with much thinner (c. 100  $\mu\text{m}$  thick) lighter colored

332 layers (Fig. 13). At least three layers were traced in the specimen, though in  
333 places the layers appear to be merged or truncated.

334

#### 335 *Specimen Caerwys 1: Microscopy*

336 A thin-section of Caerwys 1 was stained with Alizarin Red S and Potassium  
337 Ferricyanide. The pink color confirmed its non-ferroan calcite composition. The  
338 central cavity (Fig. 13) of this specimen is highly porous, evidenced by the blue  
339 resin (Fig. 14), and divided into several empty pockets by walls of sparry calcite.  
340 One empty pocket is lined with 20  $\mu\text{m}$  diameter microspar crystals (Fig. 14a) that  
341 are partially intergrown and form a porous network. On both sides of this initial  
342 cavity fill are crystal fans of sparry calcite, with crystal lengths of 80 to 150  $\mu\text{m}$ .  
343 Adjacent to the sparry calcite fans at the top of the specimen is a second empty  
344 pocket, 3 mm across, that was progressively filled by 300  $\mu\text{m}$  diameter sparry  
345 calcite fans (Fig. 14b). These grew inwards from all sides toward the cavity  
346 center. Dark-colored inclusions within some of these fans are oriented along  
347 crystallite boundaries (Fig. 14c and 14d). The origin of these filamentous  
348 microfossil-like inclusions is discussed further below. The bulk of the specimen is  
349 comprised of layers of columnar sparry calcite that grew primarily outward as  
350 fans (Fig. 14e-g). Each crystal is c. 500  $\mu\text{m}$  long and 50 to 100  $\mu\text{m}$  wide. These  
351 sparry layers correspond to the thick, lighter colored layers observed in hand

specimen that are separated by laminae of micrite c. 130  $\mu\text{m}$  thick (Fig. 13 and Fig. 14f). There is evidence that columnar sparry calcite fans were partially dissolved prior to or during deposition of the micrite layers (Fig. 14e), as some of the micrite layers are a little thicker (c. 230  $\mu\text{m}$ ) and more porous, particularly one layer close to the outside of the specimen (Fig. 14g).

## Discussion

The criteria of Sugitani et al. (2007) and Wacey (2009) developed to establish the biogenicity of potential microbial fossils were applied to evaluate the biogenicity of the fossil-like objects in the two samples studied. For these specimens, their Quaternary age and sedimentary origins are not in doubt. These rocks have never been buried to any significant depth, so the characteristics of the precipitates and the fabrics were developed in the original sedimentary depositional environment.

### *Biotic vs abiotic growth of Specimen S3.2*

A complex growth history of Specimen S3.2 was unravelled on the basis of the petrographic analysis, with different growth zones exhibiting different degrees of biotic influence. The oldest part of the specimen (in Zone 1; Figs. 5a and b) comprises dense, white colored calcitic tufa that contains clusters of hollow

371 carbonaceous tubes of fossilized cyanobacterial trichomes preserved inside  
372 sheaths. Zone 1 was formed by calcification of filamentous cyanobacterial shrubs  
373 that coated an overhanging leaf or twig. This photosynthesizing biofilm formed a  
374 substrate for subsequent growth that was necessarily focused away from the tufa  
375 wall toward the direction of light. Such biologically enhanced growth resulted in  
376 the asymmetrical, elongated form of the specimen. A network of 250 to 500  $\mu\text{m}$   
377 diameter hollow calcite tubes was formed by chironomid insect larvae, perhaps in  
378 the late Spring season as waters warmed up (Janssen et al., 1999; Brasier et al.,  
379 2011). Filamentous microbes coated these tubes, separated by at least one and  
380 possibly two or three pauses of unknown duration (perhaps weeks or months,  
381 likely at most a few years). Zones 1 and 2 are at least part contemporaneous, with  
382 Zone 2 sparry calcite shrubs containing thinner entombed carbonaceous filaments  
383 dominantly forming on the side of the specimen that received less light. Their  
384 presence on the more illuminated side of the specimen toward the end of Zone 1  
385 deposition, including in small crevices, is consistent with the onset of shaded,  
386 lower light intensity conditions that supported the growth of microbial species  
387 associated with spar formation.

388

389 The Zone 1 microfossils must have been syngenetic with the calcite deposition, as  
390 the calcite initially developed as tubes nucleated on the filaments (Figs. 4, 5, 6a

391 and 7). It is possible that the Zone 2 microfossils (Fig. 8) represent endoliths that  
392 bored into the tufa post-deposition, as they are oriented parallel to crystal growth  
393 planes and cut across the micron-scale growth lines within the sparry calcite  
394 crystal fans, features previously used as indicative of an endolithic habit (Knoll et  
395 al., 2013). However the filaments in the Caerwys samples protrude across laminae  
396 that likely formed on diurnal timescales, well within the lifetime of entombed  
397 individual microorganisms. Further, endolithic organisms would also be expected  
398 to target earlier formed tufa micrite as well as the crystal fans. The occurrence of  
399 the Zone 2 filaments close to the center of the specimen and not in the outer parts  
400 makes it unlikely that the cyanobacterial filaments preserved in Zone 2 were  
401 endoliths. More likely, and as Pedley (1987) inferred, the filaments were  
402 fossilized by syn-depositional calcite entombment.

403

404 Evidence for microbial influence on the growth of Zones 1 and 2, consistent with  
405 the microfossil biogenicity criteria of Wacey (2009), includes:

- 406 1) Two different populations of carbonaceous filaments, each community  
407 associated with its own characteristic carbonate rock microfacies and not  
408 randomly distributed.
- 409 2) Entombed clusters of hollow brown-colored carbonaceous filaments that  
410 are syngenetic with the carbonate rock. These microfossils include

411 sheathed trichomes and are comparable in size and morphology with  
412 extant cyanobacteria.

413 3) Calcitic molds of colonies of microbial filaments preserved in the  
414 carbonate rock, with calcification specifically on and around the filaments,  
415 consistent with microbial influence on crystal nucleation.

416 Zone 3 (Fig. 9) lacks the microfossils of Zones 1 and 2 (Figs. 6, 7 and 8). The  
417 thicker layering on the undersides of overhangs (Fig. 4b, 5b) is consistent with a  
418 dominantly abiotic growth process. However, as in Zone 1, the 100 to 240  $\mu\text{m}$   
419 diameter tubes (Fig. 10) are best interpreted as chironomid larval tubes (e.g.,  
420 Brasier et al., 2011). Chironomid larvae are detrital feeders, which implies that  
421 Zone 3 formed in detritus-rich flowing stream water. Tubes at the top of Zone 3  
422 could be directly associated with the micrite of Zone 4. This inference is based on  
423 the micritic peloids that could be of fecal origin, and additional tubes of likely  
424 chironomid origin that occur in the Zone 4 micrite. This suggests that Zone 3 spar  
425 and Zone 4 micrite are partly contemporaneous, and indeed Zone 4 micrite  
426 merges laterally into sparry calcite.

427 Petrographic evidence that Zone 5 columnar spar is primary and not the result of  
428 recrystallization includes the aggradational fabrics that surround the micrite  
429 inclusion (Fig. 11). That the micrite inclusion sits flat on a thin micritic layer (Fig.  
430 11a) is consistent with the latter representing a phase (perhaps a dry summer)



431 during which little calcite crystal growth occurred. New columnar crystals  
432 nucleated on top of the micrite (Fig. 11b), presumably during a wetter phase. The  
433 resulting topography caused deflection of the subsequent growth laminae.  
434 Ultimately the spar that nucleated on top of the micrite inclusion was out  
435 competed by the surrounding larger columnar crystals (Fig. 11b). Columnar spar  
436 of Zone 5 likely formed within or in very close proximity to the active tufa-  
437 depositing stream. These conditions could have been found behind the  
438 downstream facing accretionary surface of an actively accumulating tufa barrage.  
439 No obvious indicators of a biogenic cause for calcite deposition were identified in  
440 Zone 5. This does not necessarily imply that nucleation of Zone 5 spar was  
441 wholly abiotic: extracellular polymeric substances produced by microbes were  
442 very likely present in the stream water, though there was no direct evidence that  
443 they contributed to the formation of Zone 5 spar.

444 Similar to the crystals of the Zone 5 spar, the crystals of Zone 6 also lacked  
445 carbonaceous microfossils (Fig. 12). The fabric of Zone 6 was reminiscent of the  
446 open dendritic speleothem texture described by Frisia and Borsato (2010). Such  
447 textures are known to form at cave entrances, in places subject to kinetic  
448 processes such as prolonged degassing phenomena. Such a scenario is consistent  
449 with Zone 6 developing as a late-stage cave speleothem-like meteoric growth  
450 within the cavern. Unlike the within-stream conditions that supported the growth

451 of Zones 1-5, Zone 6 developed when the tufa stream system itself had become  
452 inactive.

453 In short, petrographic observations revealed that Zones 1 and 2 formed in the  
454 presence of microbes that included cyanobacteria; Zone 3 calcite growth was  
455 dominantly abiotic except for calcite tubes most likely constructed by chironomid  
456 larvae; Zone 4 is attributed to chironomid larvae that consumed microbes; there  
457 are no obvious preserved indicators of biological activity or control on calcite  
458 precipitation in Zone 5 and Zone 6, and the latter formed in different  
459 environmental circumstances compared to those that supported the growth of  
460 Zones 1 – 5. Abundant petrographic evidence for syn-depositional growth of  
461 columnar calcite spar is found in places with entombed cyanobacterial filaments  
462 (Zone 2), where crystal growth continued behind the aggrading outer surface layer  
463 (Fig. 6); where there is evidence of micrite bands capping spar layers, and  
464 competitive crystal growth of spar crystals (Fig. 9); where insect larval tubes are  
465 found in sparry calcite layers (Fig. 10); and where detrital micrite stuck to the  
466 specimen surface and interfered with growth of overlying spar crystals (Fig. 11).  
467 There is no evidence for recrystallization that would have required dissolution.  
468 Despite the presence of cyanobacteria in Zones 1 and 2, the most clearly  
469 laminated (i.e. “stromatolitic”) zones are 3 and 5 (Figs. 4 and 5). The latter are the  
470 most cave speleothem-like and arguably abiotic sections. This lamination is  
471 discussed further below.

472

473 *Biotic vs abiotic character of Specimen Caerwys 1*

474 Specimen Caerwys 1 is similar to specimen S3.2 in that it was nucleated on a  
475 downward hanging biological substrate, overgrown by successive layers of sparry  
476 calcite that extend to the outside of the sample. Caerwys 1 is a thinner specimen  
477 than S3.2. This may be partly due to absence of prograding cyanobacterial  
478 filaments in the core of Caerwys 1. Rather, the initial biological substrate seems  
479 to have been a stem or branch of a larger plant. The predominantly sparry calcite  
480 walls of Caerwys 1 are similar to Zone 2 of S3.2 (compare Figs 6b and 8 with Fig.  
481 14c-f). Clusters of potential microfossil filaments in Zone 2 of specimen S3.2  
482 (Fig. 8) and in the sparry calcite fans of Caerwys 1 (Fig. 14c) also share similar  
483 properties of size, coloring and orientation parallel to the direction of crystal  
484 growth. However, the orientation of the dark-colored Caerwys 1 filaments was  
485 directly related to the orientation of the crystal structure. A comparison of the  
486 observations against the biogenicity criteria of Sugitani et al. (2007) and Wacey  
487 (2009) indicate that the Caerwys 1 filaments are much less convincing as  
488 microfossil candidates than those found in the S3.2 sample.

489

490 *Biogenicity of the lamination?*

491 The specimens described here are layered carbonate rocks that by some  
492 definitions could be identified as “tufa stromatolites” (Riding, 1991) or simply  
493 stromatolites (e.g., Semikhatov et al., 1979). Therefore, to evaluate whether the  
494 Caerwys tufa layering would be identified as biogenic when commonly used  
495 stromatolite biogenicity criteria are applied, we used the criteria of Buick et al.  
496 (1981) and Hofmann (2000) as critiqued in McLoughlin et al. (2013).

497 The first group of biogenicity criteria pertain to the context of the lamination and  
498 include, for example, requirements that the structures be found in sedimentary or  
499 metasedimentary rocks and be syn-sedimentary with the deposit in which they are  
500 found. This is undoubtedly the case for these Quaternary tufas. Similarly, there is  
501 a criterion that brecciated mat chips should be found accumulated in depressions  
502 between convexly laminated mounds. Eroded chips of layered carbonate are  
503 found within the pool facies at Caerwys (Pedley, 1987). Such findings establish  
504 that the layered carbonate rocks are a primary sedimentary feature, but these  
505 contextual criteria do not differentiate biogenic from abiotic stromatolites.

506

507 The second group of biogenicity criteria pertain to the morphology of the  
508 lamination: a biogenic stromatolite should exhibit a preponderance of convex-  
509 upward structures; contain laminae that thicken over the crests of flexures; consist  
510 of laminations that are wavy, crinkled or have several orders of curvature; and

511 may be associated with thin, rolled-up fragments with coherent flexible laminae  
512 that can reasonably be interpreted as microbial mats. However, none of these  
513 criteria is itself diagnostic of a biogenic rock. Here we highlight, for example, that  
514 several tufa stromatolites of cyanobacterial origin exhibit isopachous layering  
515 (e.g., Janssen et al., 1999; Andrews and Brasier, 2005; Brasier et al., 2010; 2011).  
516 Spar layers in Caeryws tufa specimen S3.2 Zone 3 that might otherwise be  
517 interpreted as abiotic thicken over the crests of flexures, as do spar layers of Zone  
518 5 (Fig. 11). Laminae of specimen Caerwys 1 seem thickest at the downward  
519 pointing tip of the specimen (Fig. 13), which is consistent with the effects of  
520 gravity and hence abiotic growth. Likewise, the thin micritic layers of Caerwys 1  
521 are crinkled and curved (Fig. 13) yet the petrographic observations suggest that  
522 these micrite bands are related to periods of exposure or non-deposition, as they  
523 cap spar fans that are partially dissolved or eroded (Fig. 14e). Despite being  
524 crinkled these micrite laminae are unlikely to be biogenic.

525

526 The third group of biogenicity criteria are those that relate to the nature of  
527 microfossils and trace fossils within the lamination. Zones 1 and 2 of Specimen  
528 S3.2 exhibit both entombed hollow carbonaceous filaments and calcitic external  
529 molds of filaments oriented perpendicular to the lamination, not parallel to it.  
530 Trace fossils of insect larvae are present within Specimen S3.2 Zone 3. It is

531 possible that these larvae were farming particular species of cyanobacteria above  
532 their tubes, and that the cyanobacteria influenced crystal growth. Alternatively the  
533 larvae might have produced their own growth-influencing organic substances  
534 (Brasier et al., 2011). However there is no evidence for microbes directly causing  
535 the lamination of Zone 3. Microfossils are notably absent in the most distinctly  
536 laminated section, Zone 5.

537 Additional biogenicity criteria require that changes in the composition of  
538 microfossil assemblages should be accompanied by morphological changes in  
539 biogenic stromatolites. Where more than one microfossil assemblage is present in  
540 the samples studied, changes in micromorphology were found in these specimens.  
541 For example, the narrow filaments of Specimen S3.2 Zone 2 are associated with  
542 sparry calcite fans (Fig. 8) whereas broader filaments in trichomes of Zone 1 are  
543 found in sub-vertically hanging tubes of calcite (Figs. 4, 5, 6 and 7).

544 The entombed microorganisms of Specimen S3.2 are also organized and clustered  
545 in a fashion consistent with colonial photoautotrophic growth. We infer that their  
546 EPS did not simply bind sediment (e.g., Gerbersdorf and Wieprecht, 2014) but  
547 that it actively assisted calcite crystal nucleation (Rogerson et al., 2008; Glunk et  
548 al., 2011). This hypothesis is supported by the petrographic evidence for  
549 preferential growth of the carbonate rock toward the most light-illuminated  
550 direction. Insect larval tubes present in the Caerwys tufa (Fig. 10) would

551 potentially meet a criterion that biogenic stromatolitic fossils must be organized in  
552 a manner that indicates trapping, binding or precipitation of sediment, though  
553 whether the insect larvae actively trapped, bound or precipitated the sediment  
554 themselves (e.g., Brasier et al., 2011) could not be discerned petrographically.

555

556 Though all of above criteria establish that the lamination is sedimentary in origin  
557 (Fig.6a) and the cyanobacterial microfossils are consistent with evidence for  
558 photoautotroph-induced specimen growth (see supplementary information S?), the  
559 cause of most of the lamination in the Caeryws specimens is related to alternating  
560 episodes of specimen growth, non-deposition and erosion (e.g., Figs. 9d, 11 and  
561 14d). Physico-chemical parameters such as stream flow rates, pH, alkalinity,  
562 saturation and temperature likely controlled the development of such lamination.  
563 Though microbially produced EPS may have exerted some influence on the  
564 laminae microstructure, direct evidence for microbial control on the lamination  
565 was absent.

566 In summary, the criteria consistent with biogenic features in the layered tufas at  
567 Caerwys are those that relate to fossils of the organisms themselves, and not  
568 those of the lamination. Without the presence of microbial fossils, it would not be  
569 possible to identify the biogenicity of the laminae. Layering alone is not

570 diagnostic of a biogenic structure in specimens like those characterized in this  
571 study.

572

### 573 **Implications for astrobiology**

574 Carbonate rocks that may be targets in the search for martian microfossils were  
575 identified by Niles et al. (2013). As with Earth's deep time stromatolites  
576 (McLoughlin et al., 2013), discriminating purely abiotic chemical sedimentary  
577 precipitates from biogenic rock structures of Mars will prove challenging. It is  
578 encouraging that simple criteria devised for ancient stromatolites and microfossils  
579 (Buick et al., 1981; Hofmann, 2000; Sugitani et al., 2007; Wacey, 2009) here  
580 enabled biogenic microfossils to be distinguished from abiogenic pseudofossils in  
581 a complex terrestrial case.

582 It is worth noting that recognition of microfossils requires detailed microscopic  
583 examination: definitively identifying biogenic structures in the examined  
584 Caerwys specimens would not have been possible at the outcrop or hand-  
585 specimen scales. Robotic exploration of Mars has already located potentially  
586 habitable streams (Grotzinger et al., 2014), structures consistent macroscopically  
587 with microbially-induced sedimentary structures have been described (Noffke,  
588 2015), and chemical signals consistent with martian life (Webster et al., 2015)



589 have been reported. Potential lessons from the Caeryws quarry, however, are that  
590 the microbial structures visible under the microscope are not readily identifiable  
591 as biogenic at the hand-specimen scale, and the nature of the laminations were  
592 such that they could not be proven as biogenic at the hand-specimen or  
593 petrographic microscope scales.

594

## 595 **Conclusions**

596 The microfossils found in specimen S3.2 display at the petrographic scale the  
597 most convincing evidence for biogenicity: the layered stalactitic rock developed  
598 as a result of the calcification of filamentous cyanobacteria and different  
599 populations of carbonaceous filaments were each associated with their own  
600 characteristic carbonate rock microfacies.. The entombed clusters of hollow  
601 filamentous carbonaceous microfossils that included sheathed cyanobacterial  
602 trichomes demonstrated that the microfossil evidence is syngenetic with the  
603 carbonate rock. The influence of biology on the morphology of the deposit was  
604 identified by the strong asymmetry of the sample fabric: preferential growth on  
605 the most highly illuminated side of the barrage was associated with the presence  
606 of photoautotrophic microbes preserved as colonies of calcitic filament molds.  
607 Though some calcite spar fans in this sample formed in association with microbial  
608 filaments, the columnar spar that formed during a latter growth stage toward the

609 outside of specimen S3.2 does not include entombed microfossils. In contrast to  
610 specimen S3.2, the filaments found in the specimen Caerwys 1 were associated  
611 with the crystal structure of the deposit, which according to the criteria of Sugitani  
612 et al. (2007) and Wacey (2009), indicate that these structures are less plausible  
613 microfossil candidates than those found in S3.2.

614

615 Petrographic evidence indicates that columnar spar is a primary fabric, and not  
616 due to secondary crystal growth. This spar lacks obvious microfossils and formed  
617 within the calcite-precipitating stream, but in poorly illuminated locations behind  
618 the barrage front. Lamination is related to episodes of growth interrupted by  
619 intervals of non-deposition and erosion. We interpret the petrographic evidence as  
620 indicative of lamination formation controlled by physico-chemical parameters.  
621 Interestingly, we found that lamination in the samples studied was not an  
622 indicator of stromatolite biogenicity.

623

## 624 **Acknowledgements**

625 Natural Resources Wales and Steven Griffiths are thanked for access to Caerwys  
626 quarry and permission to work on the site. Sebastiaan Edelman and Thomas  
627 Logeman assisted with fieldwork and provided some of the field photographs.

628 Bouke Lacet (Sedimentology laboratory, VU University Amsterdam) prepared the  
629 thin-sections. Three anonymous reviewers helped to sharpen the manuscript, and  
630 Sherry Cady provided valuable editorial advice and assistance. ATB was inspired  
631 by Martin Brasier. He dedicates this manuscript to his father's memory.

632

### 633 **Author Disclosure Statement**

634 No competing interests exist.

635

### 636 **References**

637 Andrews, J.E., Brasier, A.T., 2005. Seasonal records of climatic change in  
638 annually laminated tufas: short review and future prospects. *Journal of*  
639 *Quaternary Science* 20: 411-421.

640 Arp, G., Wedemeyer, N., Reitner, J., 2001. Fluvial tufa formation in a hard-  
641 water creek (Deinschwanger Bach, Franconian Alb, Germany). *Facies* 44: 1-22.

642 Brasier, A.T., 2011. Searching for travertines, calcretes and speleothems in deep  
643 time: Processes, appearances, predictions and the impact of plants. *Earth-Science*  
644 *Reviews* 104: 213-239.

645 Brasier, A.T., 2007. Palaeoenvironmental Reconstructions from Quaternary Tufas  
 646 and Calcretes, central Greece. Unpublished Ph. D. thesis, University of East  
 647 Anglia, Norwich, UK. 294p.

648 Brasier, A.T., Andrews, J.E., Marca-Bell, A.D., Dennis, P.F. 2010. Depositional  
 649 continuity of seasonally laminated tufas: Implications for  $\delta^{18}\text{O}$  based  
 650 palaeotemperatures. *Global and Planetary Change* 71: 160-167.

651 Brasier, A.T., Andrews, J.E., Kendall, A.C., 2011. Diagenesis or diagenesis?  
 652 The origin of columnar spar in tufa stromatolites of central Greece and the role of  
 653 chironomid larvae. *Sedimentology* 58: 1283-1302.

654 Buick, R., Dunlop, J., Groves, D., 1981. Stromatolite recognition in ancient rocks:  
 655 an appraisal of irregularly laminated structures in an Early Archaean chert-barite  
 656 unit from North Pole, Western Australia. *Alcheringa* 5: 161-181.

657 Cacchio, P., Contento, R., Ercole, C., Cappuccio, G., Martinez, M.P., Lepidi, A.,  
 658 2004. Involvement of microorganisms in the formation of carbonate speleothems  
 659 in the Cervo Cave (L'Aquila-Italy). *Geomicrobiology Journal* 21: 497-509.

660 Fairchild, I. J., Smith, C. L., Baker, A., Fuller, L., Spötl, C., Matthey, D., &  
 661 McDermott, F. (2006). Modification and preservation of environmental signals in  
 662 speleothems. *Earth-Science Reviews* 75: 105-153.

663 Freytet, P., Verrecchia, E.P., 1998. Freshwater organisms that build stromatolites:  
664 a synopsis of biocrystallization by prokaryotic and eukaryotic algae.  
665 *Sedimentology* 45: 535-563.

666 Frisia, S., Borsato, A., 2010. Karst. In *Carbonates in Continental Settings: Facies,*  
667 *Environments, and Processes* edited by Alonso-Zarza, A.M. and Tanner, L.H.,  
668 *Developments in Sedimentology* 61. Elsevier, Amsterdam.

669 Garnett, E., Andrews, J., Preece, R., Dennis, P., 2006. Late-glacial and early  
670 Holocene climate and environment from stable isotopes in Welsh tufa.  
671 *Quaternaire* 17: 31 - 42.

672 Gerbersdorf, S.U., Wieprecht, S. 2014. Biostabilization of cohesive sediments:  
673 revisiting the role of abiotic conditions, physiology and diversity of microbes,  
674 polymeric secretion, and biofilm architecture. *Geobiology* 13: 68-97.

675 Glunk, C., Dupraz, C., Braissant, O., Gallagher, K.L., Verrecchia, E.P., Visscher,  
676 P.T., 2011. Microbially mediated carbonate precipitation in a hypersaline lake,  
677 Big Pond (Eleuthera, Bahamas). *Sedimentology* 58: 720-736.

678 Grotzinger, J.P., Sumner, D.Y., Kah, L.C., Stack, K., Gupta, S., Edgar, D. et al.,  
679 2014. A habitable fluvio-lacustrine environment at Yellowknife Bay, Gale Crater,  
680 Mars. *Science* 343 (6169). doi: 10.1126/science.1242777

681 Niles, P.B., Catling, D.C., Berger, G., Chassefiere, E., Ehlmann, B.L., Michalski,  
682 J.R., Morris, R., Ruff, S.W., Sutter, B. 2013. Geochemistry of carbonates on  
683 Mars: Implications for climate history and nature of aqueous environments. *Space*  
684 *Science Reviews* 174: 301-328.

685 Hammer, Ø., Dysthe, D.K., Jamtveit, B., 2010. Travertine terracing: patterns and  
686 mechanisms. *Geological Society, London, Special Publication* 336: 345-355.

687 Hofmann, H., 2000. Archean stromatolites as microbial archives. In *Microbial*  
688 *Sediments*. edited by Awramik, S.A., and Riding, R. Springer Berlin Heidelberg,  
689 pp. 315-327.

690 Janssen, A., Swennen, R., Podoor, N., Keppens, E., 1999. Biological and  
691 diagenetic influence in Recent and fossil tufa deposits from Belgium. *Sedimentary*  
692 *Geology* 126: 75-95.

693 Jones, B., 2010. Microbes in caves: agents of calcite corrosion and precipitation.  
694 *Geological Society, London, Special Publication* 336: 7-30.

695 Jones, B., Peng, X., 2012. Intrinsic versus extrinsic controls on the development  
696 of calcite dendrite bushes, Shuzhishi Spring, Rehai geothermal area, Tengchong,  
697 Yunnan Province, China. *Sedimentary Geology* 249: 45-62.

698 Kendall, A.C., Broughton, P.L., 1978. Origin of fabrics in speleothems composed  
699 of columnar calcite crystals. *Journal of Sedimentary Petrology* 48: 519-538.

700 Knoll, A.H., Worndle, S, and Kah, L.C., 2013. Covariance of microfossil  
701 assemblages and microbialite textures across an upper Mesoproterozoic carbonate  
702 platform. *Palaios* 28: 453-470.

703 Love, K.M., Chafetz, H.S., 1988. Diagenesis of laminated travertine crusts,  
704 Arbuckle Mountains, Oklahoma. *Journal of Sedimentary Research* 58: 441-445.

705 McLoughlin, N., Melezhik, V.A., Brasier, A.T., Medvedev, P.V. 2013.  
706 Palaeoproterozoic stromatolites from the Lomagundi-Jatuli interval of the  
707 Fennoscandian Shield. In: Melezhik, V.A. et al. (eds) Reading the Archive of  
708 Earth's Oxygenation Volume 3: Global Events and the Fennoscandian Arctic  
709 Russia – Drilling Early Earth Project. *Frontiers in Earth Sciences* 3:1298-1351.  
710 Springer-Verlag, Berlin. doi: 10.1007/978-3-642-29670-3\_8

711 Noffke, N., 2009. The criteria for the biogenicity of microbially induced  
712 sedimentary structures (MISS) in Archean and younger sandy deposits. *Earth-*  
713 *Science Reviews* 96: 173-180.

714 Noffke, N., 2015. Ancient sedimentary structures in the <3.7 Ga Gillespie Lake  
715 Member, Mars, that resemble macroscopic morphology, spatial associations, and  
716 temporal succession in terrestrial microbialites. *Astrobiology* 15: 169-192.

717 Pedley, H., 1987. The Flandrian (Quaternary) Caerwys tufa, North Wales: an  
 718 ancient barrage tufa deposit. *Proceedings of the Yorkshire Geological Society* 46:  
 719 141-152.

720 Pedley, H.M., 1990. Classification and environmental models of cool freshwater  
 721 tufas. *Sedimentary Geology* 68: 143-154.

722 Pedley, M., 2014. The morphology and function of thrombotic calcite  
 723 precipitating biofilms: A universal model derived from freshwater mesocosm  
 724 experiments. *Sedimentology* 61: 22-40.

725 Pedley, M., Rogerson, M., Middleton, R., 2009. Freshwater calcite precipitates  
 726 from *in vitro* mesocosm flume experiments: a case for biomediation of tufas.  
 727 *Sedimentology* 56: 511-527.

728 Preece, R.C., 1978. The biostratigraphy of Flandrian tufas in southern Britain.  
 729 Unpublished PhD thesis, University of London.

730 Preece, R.C., Turner, C., Green, H., 1982. Field excursion to the tufas of the  
 731 Wheeler Valley and to Pontnewydd and Cefn caves. Quaternary Research  
 732 Association Field Guide, London.

733 Preece, R., Turner, C., 1990. The tufas at Caerwys and Ddol. *North Wales Field*  
 734 *Guide* 162-166.



735 Rasmussen, B., Blake, T.S., Fletcher, I.R., Kilburn, M.R., 2009. Evidence for  
 736 microbial life in syngedimentary cavities from 2.75 Ga terrestrial environments.  
 737 *Geology* 37: 423-426.

738 Riding, R., 1991. Classification of microbial carbonates. In *Calcareous Algae and*  
 739 *Stromatolites*. Springer-Verlag, Berlin.

740 Rogerson, M., Pedley, H.M., Wadhawan, J.D., Middleton, R., 2008. New insights  
 741 into biological influence on the geochemistry of freshwater carbonate deposits.  
 742 *Geochimica et Cosmochimica Acta* 72: 4976-4987.

743 Rogerson, M., Pedley, H., Kelham, A., Wadhawan, J., 2014. Linking  
 744 mineralisation process and sedimentary product in terrestrial carbonates using a  
 745 solution thermodynamic approach. *Earth Surface Dynamics* 2: 197-216.

746 Sugitani, K., Grey, K., Allwood, A., Nagaoka, T., Mimura, K., Minami, M.,  
 747 Marshall, C.P., Van Kranendonk, M.J., and Walter, M.R., 2007. Diverse  
 748 microstructures from Archaean chert from the Mount Goldsworthy–Mount Grant  
 749 area, Pilbara Craton, Western Australia: microfossils, dubiofossils, or  
 750 pseudofossils? *Precambrian Research* 158: 228-262.

751 Wacey, D., 2009. Early Life on Earth: A Practical Guide. Springer.

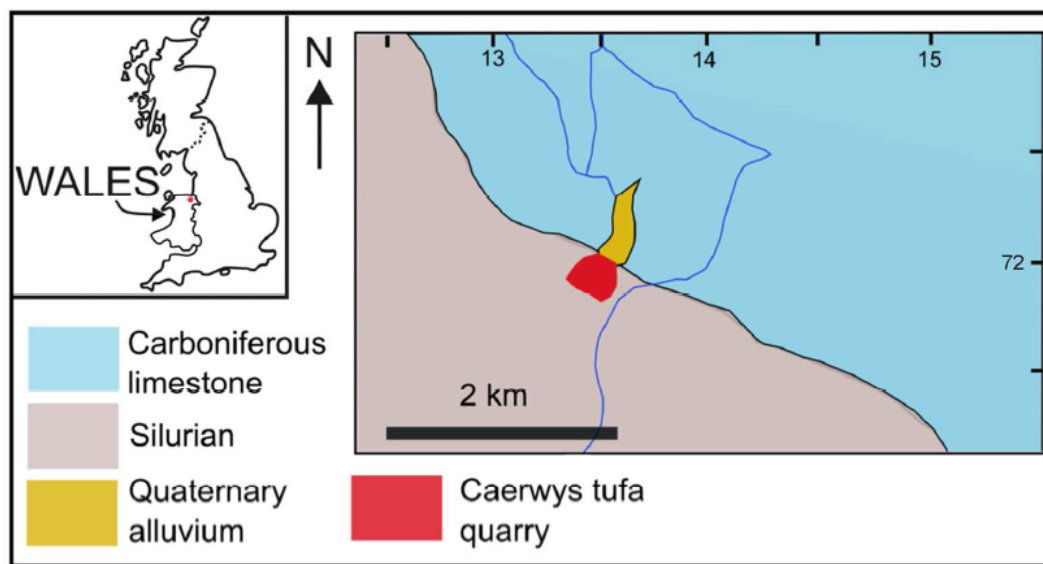
752 Webster, C. R., Mahaffy, P.R. Atreya, S.K., Flesch, G.J., Mischna, M.A., et al.  
 753 Mars methane detection and variability at Gale crater. *Science* 347: 415-417.

754 Wright, V.P., Barnett, A.J., 2015. An abiotic model for the development of  
 755 textures in some South Atlantic early Cretaceous lacustrine carbonates. In  
 756 Bosence, D.W.J. et al. (eds) Microbial carbonates in space and time: Implications  
 757 for global exploration and production. Geological Society, London, Special  
 758 Publications 418, doi: 10.1144/SP418.3

759 Zhang, D.D., Zhang, Y., Zhu, A., Cheng, X., 2001. Physical Mechanisms of River  
 760 Waterfall Tufa (Travertine) Formation. *Journal of Sedimentary Research* 71: 205-  
 761 216.

762

763 **Figure captions**



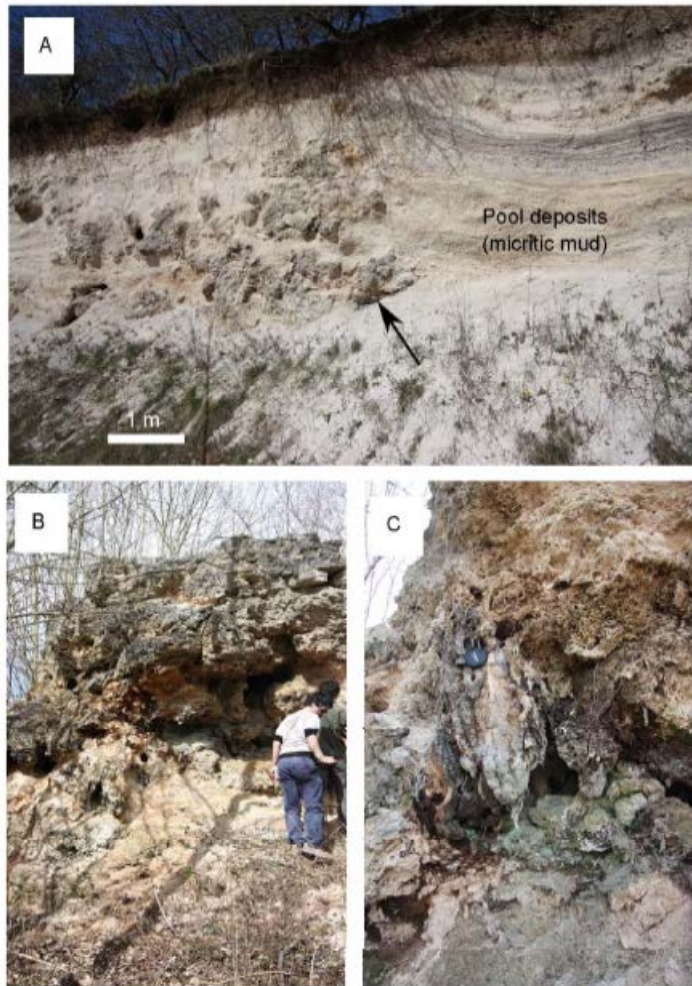
764

765 **FIG. 1:** Map showing the location of the Caerwys tufa in the UK. Inset map

766 shows the UK, with Wales labelled and Caerwys as a red dot. The larger map

767 shows the location of the Caerwys tufa quarry (red), and its relationship to  
768 surrounding Quaternary alluvial sediments (orange) and underlying geology.  
769 Carboniferous limestone (blue) supplies the calcium and bicarbonate ions to  
770 groundwaters. These precipitate tufa calcite on emergence at springs associated  
771 with the contact between the limestone and underlying Silurian siliciclastic rocks  
772 (grey). Scale bar is 2km.

773



774

775 **FIG. 2:** Caerwys tufa quarry photographs **(a)** Unconsolidated micritic pool  
 776 deposits on the right side of the image, adjacent to consolidated barrage facies  
 777 (arrow points to the base of the barrage). The tufa barrage prograded downstream  
 778 (towards the left). Isolated thrombolitic heads are found in the pool facies. **(b)**  
 779 Block of the main tufa barrage preserved in the quarry center. People for scale are  
 780 c.1.75m tall. **(c)** Stalactitic carbonate rocks that grew in a cavity of the same

781 barrage block shown in (b). Specimen Caerwys 1 is from this location, and S3.2  
782 was found as a float specimen nearby. Lens cap for scale is 5.5 cm diameter.

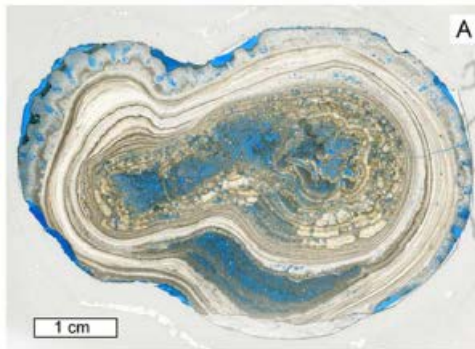
783



784

785 **FIG. 3:** Active tufa stream at Alport, Derbyshire, UK, as an analogue for the  
786 Quaternary Caerwys tufa. **(a)** View showing pool environment behind a barrage  
787 (to the right of this image), with a thrombolitic head (see arrow). The white water  
788 upstream is flowing over a barrage. **(b)** Close-up of a barrage, showing microbial

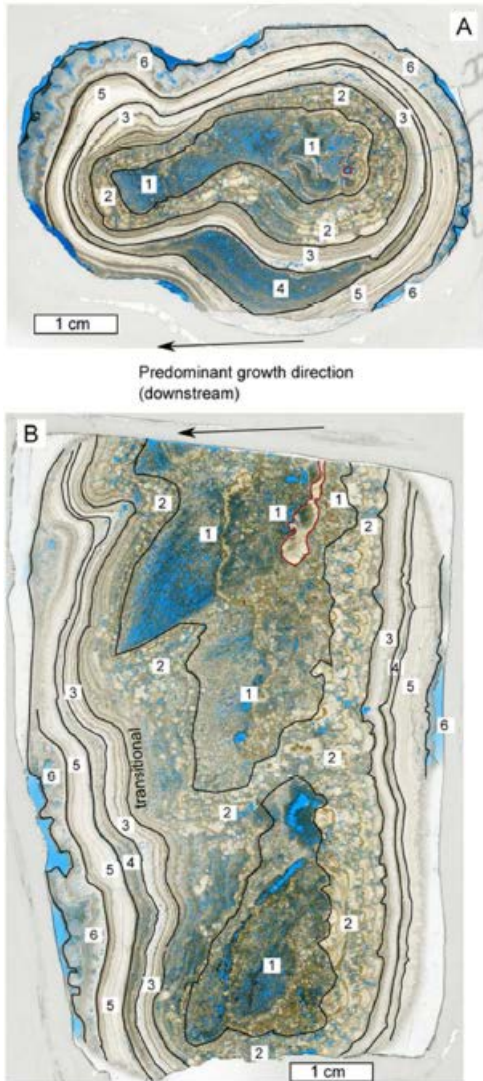
789 biofilm plus some green algae and bryophytes living subaqueously on the barrage  
790 front, hanging down into the fast flowing stream. A cavity is developing behind  
791 this calcifying structure sometimes referred to as a curtain. In larger barrages,  
792 meter-scale caverns form behind the curtain (e.g., fig. 2c).



793

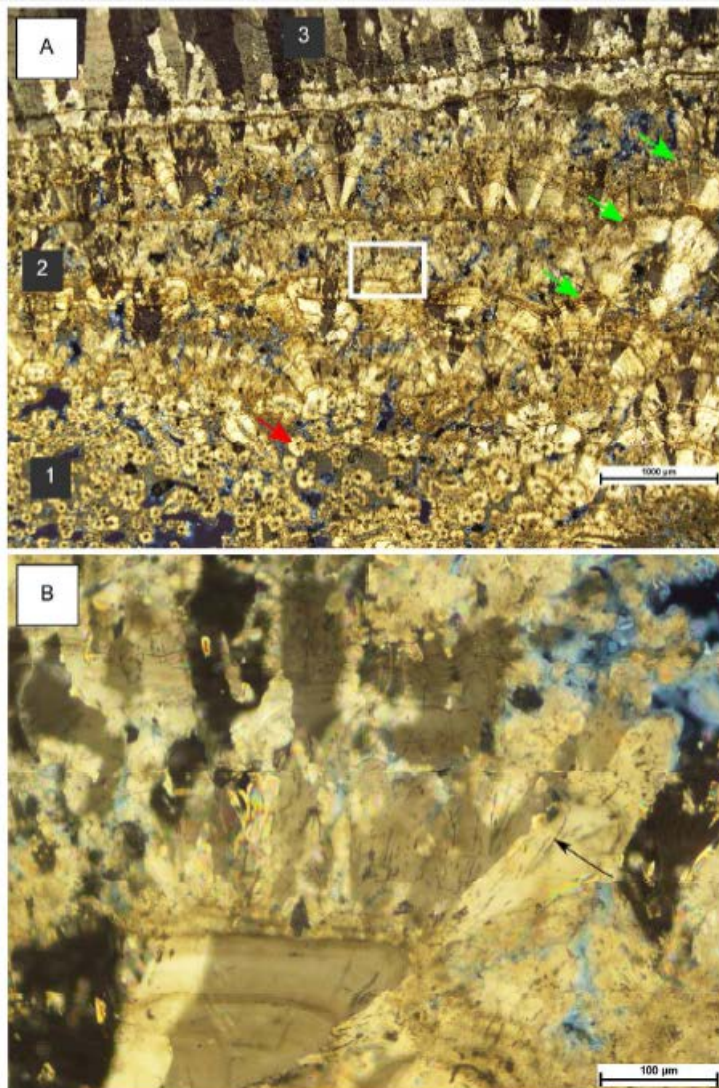


794 **FIG. 4:** Scans of thin-sections of specimen S3.2. (a) transverse cut, and (b)  
 795 longitudinal cut. Blue color is from resin injected to show porosity.



796  
 797 **FIG. 5:** Interpreted scans of thin-sections of specimen S3.2. (a) transverse cut and  
 798 (b) longitudinal cut. Interpreted boundaries between growth zones are shown as  
 799 black lines, and the position from which growth initiated is shown as a red line.

800 Zones 1-6 are described in the text. Unlike wholly abiotic stalactites, this  
801 specimen is strongly asymmetrical, caused by calcifying cyanobacteria favoring  
802 the illuminated side of the specimen. Images are shown at the same scale.

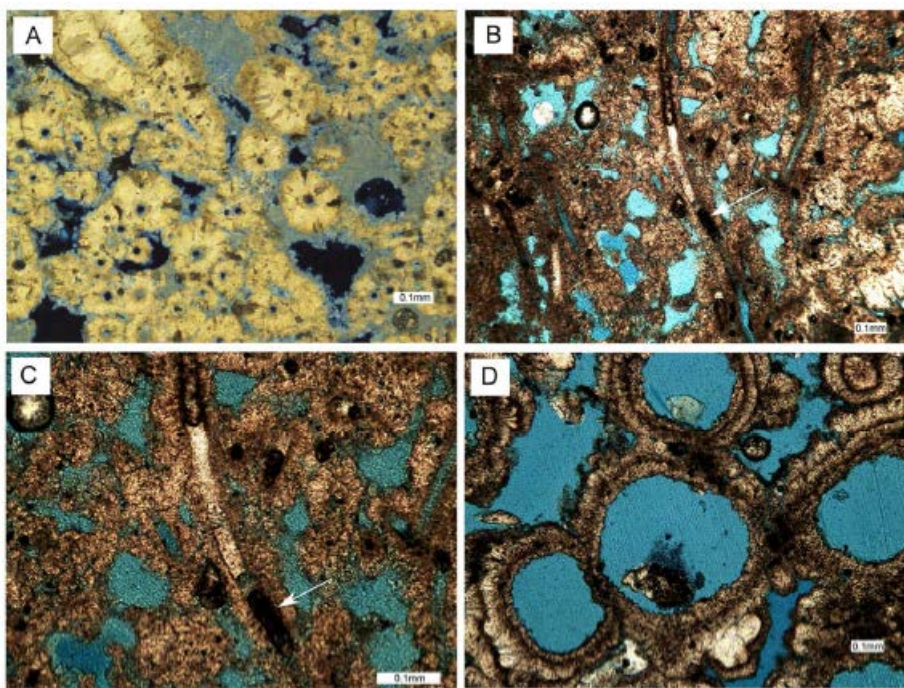


803  
804 **FIG. 6:** Micrographs of Specimen S3.2 thin-section A (transverse cut). (a) shows  
805 Zone 1 (base, hollow calcite tubes that formed around downward-hanging



806 cyanobacterial filaments; example arrowed), Zone 2 (center, sparry calcite fans  
807 interlayered with micritic bands) and Zone 3 (top, columnar calcite crystals).  
808 Numbered squares refer to these zones. Green arrows point to cessations in  
809 growth, marked by dark micritic layers, from which new fans nucleated. White  
810 box in Zone 2 shows location of (b), which is a sparry calcite fan that contains  
811 entombed cyanobacterial filaments (arrowed).

812



813

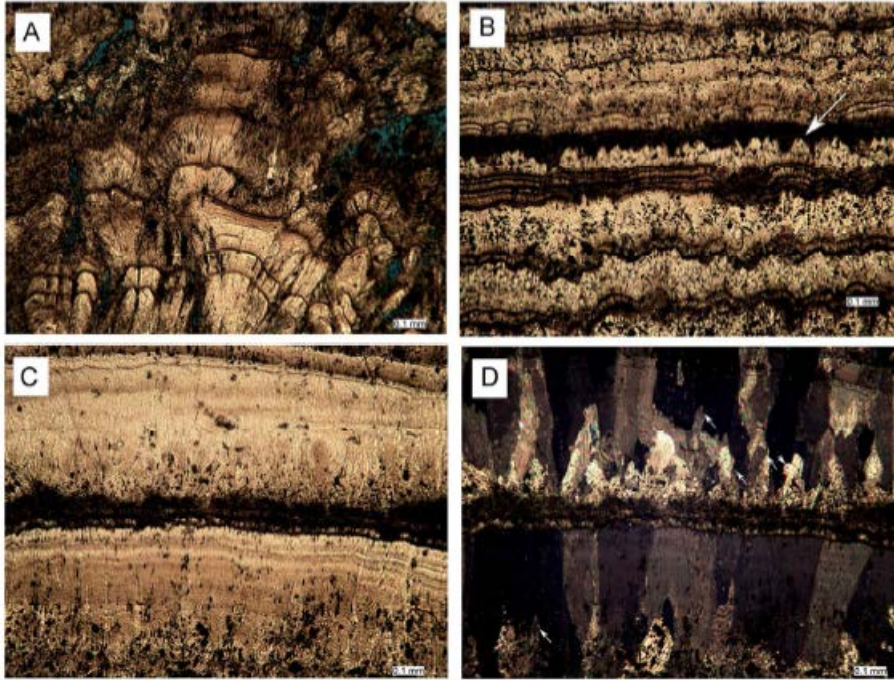
814 **FIG. 7:** Micrographs of Specimen S3.2 showing biological fabrics. (a) Cross-  
815 polarized light image of Zone 1 calcite tubes formed around downward-hanging  
816 cyanobacterial filaments (transverse cut). Blue is resin, and black is holes in the

817 thin-section. **(b)** Plane polarized light image of calcite tubes containing  
818 microfossils of filamentous cyanobacteria (longitudinal cut). Trichome in a sheath  
819 is arrowed. **(c)** Higher magnification plane polarized light image of the trichome  
820 and sheath shown in (b). **(d)** Plane polarized light image of a network of larger  
821 diameter calcite-cemented holes best interpreted as insect (interpreted as  
822 chironomid?) larval tubes.



823  
824 **FIG. 8:** A sparry calcite fan containing entombed cyanobacterial filaments of  
825 specimen S3.2 Zone 2. Note the filaments are dominantly oriented parallel to the  
826 direction of crystal growth. However they also curve and cross each other, and are  
827 not artifacts related to the crystal structure. Filaments also cross dark colored

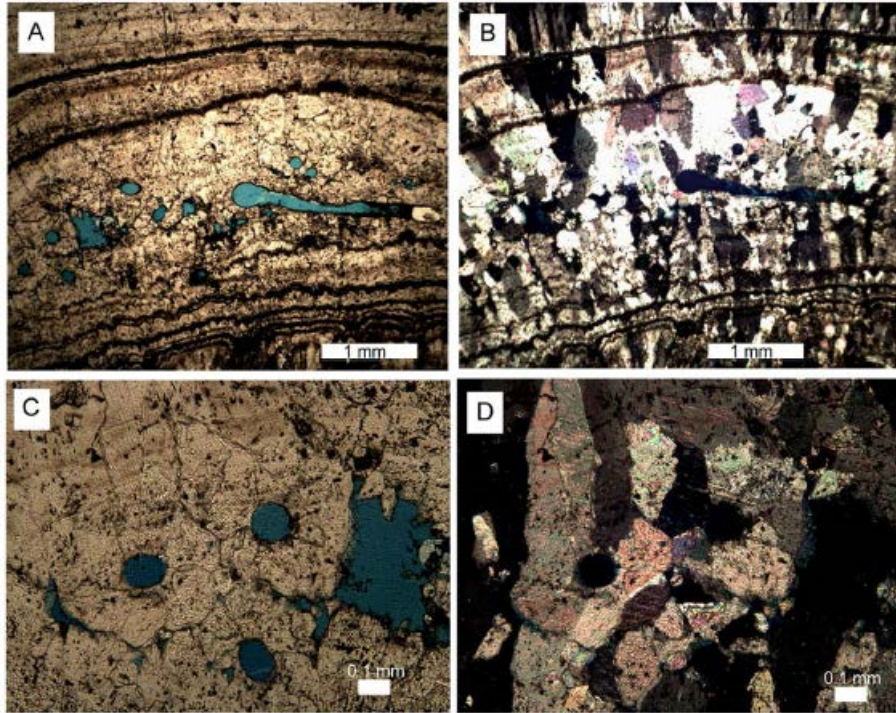
828 growth banding (likely formed on ~diurnal timescales) in the crystal. Scale bar is  
829 0.1 mm across.



830  
831 **FIG. 9:** Sparry calcite of Specimen 3.2 Zone 3. **(a)** shows the top of Zone 2,  
832 transitioning into Zone 3. Note the shrub-like calcite crystal fans with entombed  
833 cyanobacterial filaments of Zone 2. **(b)** shows alternating laminae of calcite spar  
834 and micrite of Zone 3. The arrow points to a partially broken pointed termination  
835 of a spar crystal draped in dark micrite. **(c)** shows (apparently abiotic) sparry  
836 calcite laminae found further from the specimen center than (b), with dusty  
837 lamination caused by inclusions. **(d)** is the same area as (c), with polars crossed.  
838 Note competitive growth has favored some crystals over others, resulting in a



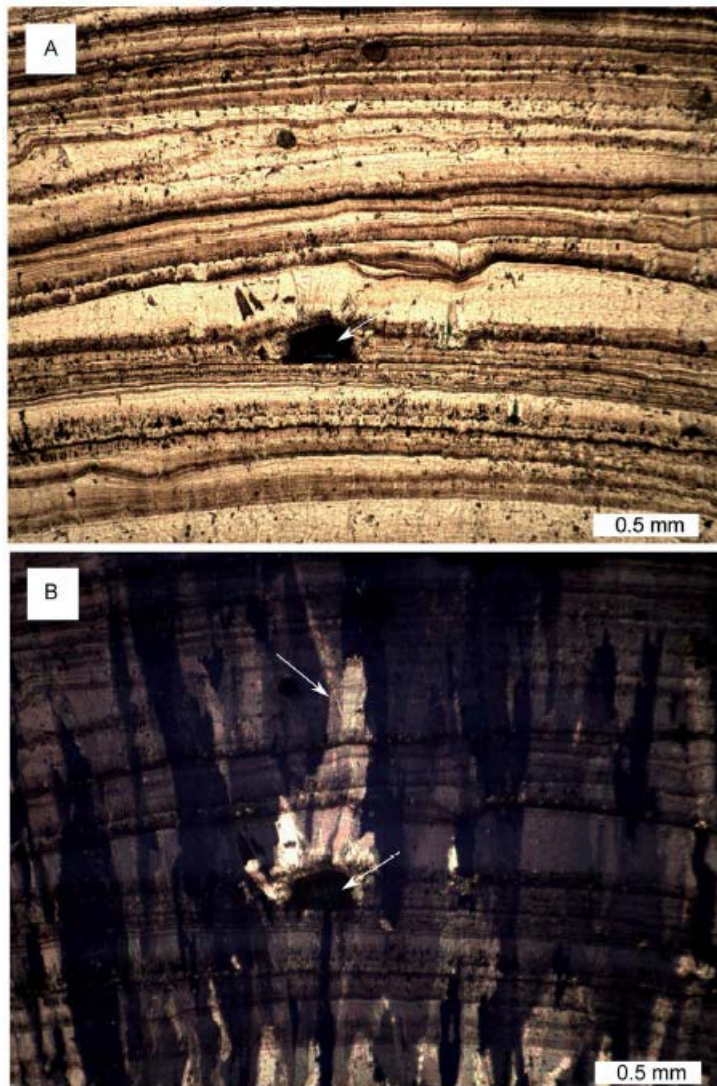
839 columnar fabric. Examples of crystals that were out-competed are arrowed. Note  
 840 also that new crystals nucleated on the micrite layers (presumed hiatuses in  
 841 growth), consistent with this columnar spar being a primary fabric.



842 **FIG. 10:** Insect (likely chironomid) larval tubes in Specimen S3.2 Zone 3 spar.  
 843 These are seen as evidence that the spar is primary and not recrystallized from  
 844 micrite. (a) plane polarized light image, showing hollow tube (filled with blue  
 845 resin) within the spar of Zone 3. (b) Same area as (a), with polars crossed. Note  
 846 the influence of the insect larvae on crystal orientations. (c) Close up of some of  
 847 the tubes in plane polarized light. (d) Same area as (c), with polars crossed. Some  
 848 crystal orientations seem consistent with the broader columnar fabric of Zone 3.  
 849

850 Crystals with different orientations might have been detrital grains assimilated by  
851 the larvae for tube construction.

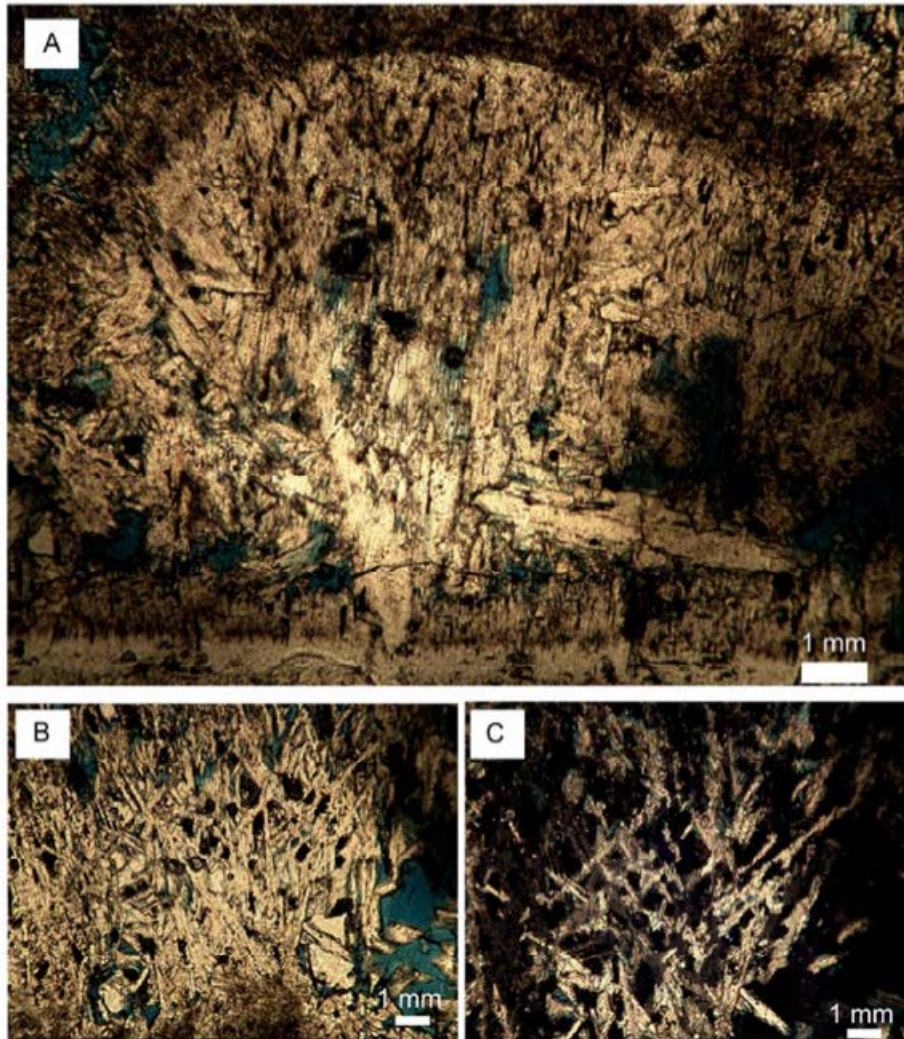
852



853

854 **FIG. 11:** Caerwys specimen 3.2 Zone 5 in thin-section. **(a)** Plane polarized light  
855 image, showing sparry crystals (light) with dark micritic growth laminae. Arrow  
856 points to a micritic grain that stuck to the specimen surface during growth, and  
857 became enveloped in (primary) columnar spar. **(b)** Same area as (a) with polars  
858 crossed. Note the influence of the detrital micrite clast (lower arrow) on columnar  
859 spar growth. Crystals nucleated on the micrite were out-competed by the larger  
860 columnar crystals (top arrow). This suggests the columnar spar was primary,  
861 despite the fact that these large crystals cut through the dusty growth lamination.  
862





863

864 **FIG. 12:** Caerwys specimen 3.2 Zone 6 in thin-section. **(a)** Plane polarized light  
 865 image showing a mound of crystal laths nucleated on a flat surface (the top of a  
 866 Zone 5 columnar calcite crystal, at the base of the image). **(b)** Close up of  
 867 network of lath-shaped crystallites in plane polarized light. **(c)** Image with polars  
 868 crossed, showing the network of lath-shaped crystallites forms composite crystals  
 869 of Zone 6. All scale bars are 1 mm.

870

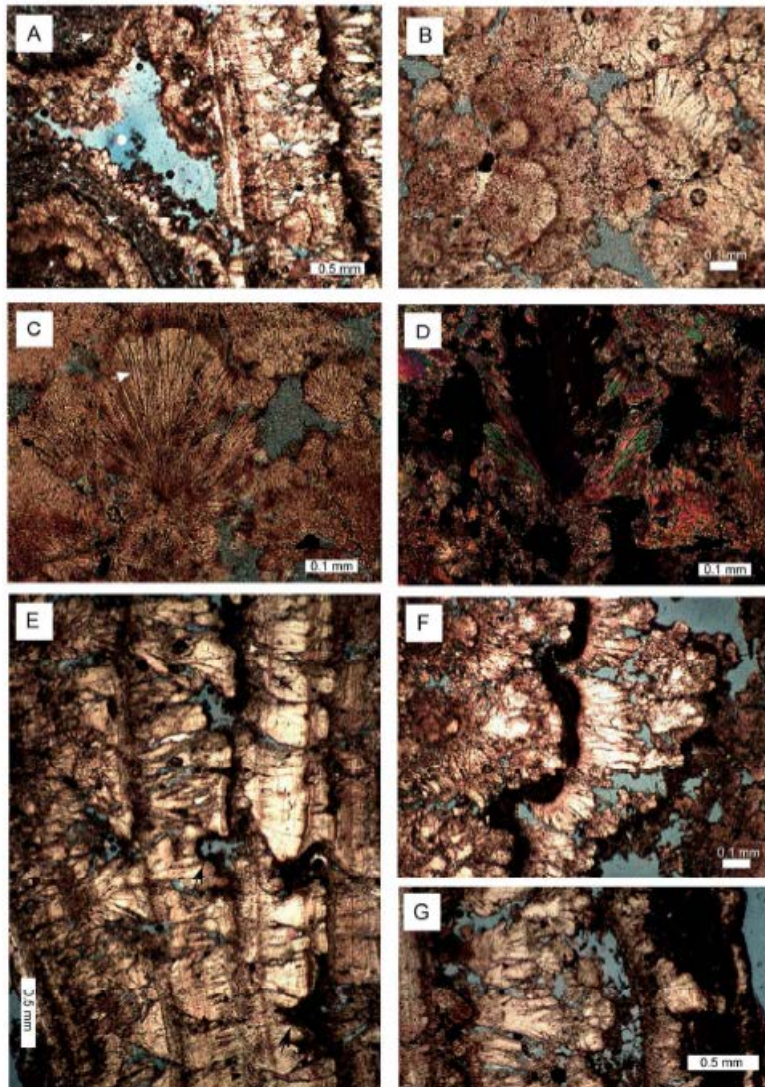


871

872 **FIG. 13:** Stalactitic specimen Caerwys 1 (longitudinally cut hand-specimen). The  
873 central cavity formed around a downward-hanging twig. Alternating micritic  
874 laminae (white) and sparry calcite fans (darker and thicker) grew on the outside.



875 Ruler for scale (larger divisions are centimeters, smaller divisions are  
876 millimeters).



877

878

879 **FIG. 14:** Photomicrographs of Caerwys 1 thin-section. **(a)** The central cavity  
880 region is highly porous, with several hollow, empty pockets (blue resin). The  
881 empty pocket in (a) is lined with 20µm diameter microspar crystals. These grew  
882 on peloidal micrite (arrowed). **(b)** A second empty pocket in the central zone, 3  
883 mm across, that has been progressively filled by 300 µm diameter sparry calcite  
884 fans. **(c)** Inclusions within these fans (arrowed), oriented along crystallite  
885 boundaries. Their alignment and form suggest they are not cyanobacterial and  
886 possibly not even microbial filaments. **(d)** Same crystal fan as shown in (c), with  
887 polars crossed. **(e)** Layers of columnar sparry calcite growing dominantly outward  
888 as fans that form the bulk of the specimen. There is evidence that columnar sparry  
889 calcite fans were partially dissolved prior to or during deposition of the micrite  
890 layers. **(f)** Close-up of one of the micritic laminae (dark band) between sparry  
891 calcite fans. **(g)** Sparry calcite fans on the left, capped by a thick micrite layer  
892 (dark band on the right) close to the outside of the specimen. All images taken in  
893 plane polarized light except (d). Scale bars in (a), (e), and (g) are 0.5mm; scale  
894 bars in other images are 0.1 mm.